IS 3827 (2008): Riboflavin, Food Grade [FAD 8: Food Additives]

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भारतीय मानक

राईबोफलेविन, खाद्य ग्रेड — विशिष्टि

(पहला पुनरीक्षण)

Indian Standard

RIBOFLAVIN, FOOD GRADE — SPECIFICATION

(First Revision)

ICS 67.220.20

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

May 2008

Price Group 2

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

Riboflavin is widely distributed in plant and animal cells, the chief sources being liver, kidney, heart, egg yolk, object yeast, dried whey, malt and dried alfalfa. It is a member of vitamin B group and is also used as a colouring matter. It is extracted from natural sources or manufactured synthetically. Riboflavin can also be prepared by submerged fermentation by *Bacillus subtilus* genetically modified for riboflavin overproduction. The strain is non-pathogenic and non-toxicogenic.

This standard is one of a series of Indian Standards for natural food colours permitted under the *Prevention of Food Adulteration Rules*, 1955.

This standard was first published in 1966 based on the then existing 'Specification for identity and purity of food additives, Vol II Food Colours' published by FAO & WHO. This standard is being revised taking into consideration the latest publication for the food colour issued by JECFA and also the latest specifications laid down under the US FDA and the EEC Directives. In this revision the limits for sulphated ash and subsidiary colouring matters as lumiflavin have been included and requirements for heavy metal contaminants made more stringent to align with the international requirements.

Requirements for riboflavin, food grade have been prescribed under the *Prevention of Food Adulteration Rules*, 1955. In the formulation of this standard, due consideration has been given to the *Prevention of Food Adulteration Act*, 1954 and the *Rules* framed thereunder. Due consideration has also been given to the *Standard of Weights & Measures (Packaged Commodities) Rules*, 1977. However, this standard is subject to restrictions imposed under these, wherever applicable.

Description

Common Name — Riboflavin

Synonyms — Vitamin B_2; lactoflovin; INS No. 101 (i)

C.A.S. Number — 83-88-5

Chemical Names — Riboflavin; 3, 10-dihydro-7, 8-dimethyl -10-[(2S, 3S, 4R)-2, 3, 4, 5-tetrahydroxypentyl] benzo-[g]pteridine-2, 4-dione; 7, 8-dimethyl-10- (1' D-ribityl) isoalloxazine.

Empirical Formula — $C_{17}H_{20}N_4O_6$

Molecular Weight — 376.37

Solubility — Very slightly soluble in water; practically insoluble in alcohol, chloroform, acetone and ether; very soluble in dilute alkali solutions.

The structural formula of riboflavin is given below:

(*Continued on third cover*)

Indian Standard

RIBOFLAVIN, FOOD GRADE — SPECIFICATION

(First Revision)

1 SCOPE

This standard prescribes requirements and methods of sampling and test for riboflavin, food grade.

2 REFERENCES

The following standards contain provisions, which through reference in this text constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
266 : 1993	Sulphuric acid (*third revision*)
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1994	Methods of sampling and test for food colours (*second revision*)
2491 : 1998	Food hygiene — General principles — Code of practice (*second revision*)

3 DESCRIPTION

Riboflavin is a yellow to orange-yellow crystalline powder, with slight odour.

4 REQUIREMENTS

4.1 Identification

4.1.1 *Spectrophotometric*

Using the aqueous solution (10 percent, *w/v*) conduct a spectrophotometric assay to determine the absorbance (A) at 257 nm, 375 nm and 444 nm. The ratio A_{375}/A_{267} is between 0.31 and 0.33. The ratio A_{444}/A_{267} shall be between 0.36 and 0.39.

4.1.2 *Specific Rotation*

Dry the sample at 100°C for 4 h. Dissolve 50.0 mg in 0.05 N sodium hydroxide (free from carbonate) and dilute to 10.0 ml with the same solvent. Measure the optical rotation within 30 min of dissolution. The specific solution shall be between 120° to 135°.

4.1.3 *Colour Reaction*

Dissolve about 1 mg of sample in 100 ml of water. The solution has a pale greenish-yellow colour by transmitted light, and by reflected light has an intense yellowish-green fluorescence, which disappears on the addition of mineral acids and alkalis.

4.2 The 'Genetic Engineering Approval Committee' of the Ministry of Environment and Forests is to be approached for granting necessary permission before release of Riboflavin manufactured from Genetically Modified (GM) strain of *Bacillus subtilis*.

4.3 The product shall also conform to the requirements given in Table 1.

Table 1 Requirements for Riboflavin, Food Grade

Sl No.	Characteristic	Requirement	Method of Test Ref to	
			Annex of This Standard	Clause of IS 1699
(1)	(2)	(3)	(4)	(5)
i)	Purity, percent by mass (on dry basis), *Min*	98	A	–
ii)	Loss on drying, percent by mass, *Max*	1.5	B	–
iii)	Sulphated ash, percent of total colouring matters, *Max*	0.1	C	–
iv)	Lumiflavin, percent by mass, *Max*	0.025	D	–
v)	Primary aromatic amines (as aniline), mg/kg, *Max*	100	–	11
vi)	Arsenic (as As), mg/kg, *Max*	3	–	15
vii)	Lead (as Pb), mg/kg, *Max* (for Riboflavin obtained from *Bacillus subtilis*)	10	–	15

4.4 The product shall be processed, packed, stored and distributed under hygienic conditions in licensed premises (*see* IS 2491).

5 PACKING, STORAGE AND MARKING

5.1 Packing

The product shall be filled in amber-coloured glass containers or any other suitable containers with as little air space as possible. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

5.2 Storage

The product shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

5.3 Marking

Each container shall be legibly and indelibly marked with the following information:

a) Name of the material including the words 'Food Grade';
b) Name and address of the manufacturer;
c) Batch or code number;
d) Net content when packed;
e) Instruction for storage;
f) Best before ..... (Month and year to be given by the manufacturer); and
g) Any other requirements as given under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977 and *Prevention of Food Adulteration Act*, 1955 and the Rules.

5.3.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

5.3.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

Representative samples of the material shall be drawn according to the method prescribed in **4** of IS 1699.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis.

ANNEX A

[*Table* 1, *Sl No.* (i)]

DETERMINATION OF PURITY

A-1 REAGENTS

A-1.1 2 N Sodium Hydroxide

A-2 APPARATUS

A-2.1 Spectrophotometer, capable of accurate (±1 percent or better) measurement of absorbance in the region of 350-750 nm with an effective slit width of 10 nm or less.

A-3 PROCEDURE

Carry out the assay in subdued light. In a brown glass 500-ml volumetric flask, suspend 65 mg of the sample in 5 ml of water, ensuring that it is completely wetted, and dissolve in 5 ml of 2 N sodium hydroxide solution. As soon as dissolution is complete, add 100 ml of water and 2.5 ml of glacial acetic acid and dilute to 500 ml with water. Place 20 ml of this solution in a brown glass 200-ml volumetric flask, add 3.5 ml of a 1.4 percent *w/v* solution of sodium acetate and dilute to 200 ml with water. Measure the absorbance (*A*) at the maximum at 444 nm.

A-4 CALCULATION

$$\text{Percent, Riboflavin} = \frac{A \times 5\ 000}{328 \times W} \times 1.367$$

where

A = absorbance of the sample solution at 444 nm; and

W = mass of sample, in g.

ANNEX B

[*Table* 1, *Sl No.* (ii)]

DETERMINATION OF LOSS ON DRYING

B-1 PROCEDURE

Weigh accurately about 2 g of the material in a tared weighing bottle fitted with a ground glass lid. A weighing bottle of squat form, about 50 mm in diameter and 30 mm in height, is suitable. Heat for 4 h in an air oven at 105°C, cool in a dessicator and weigh.

B-2 CALCULATION

$$\text{Loss on drying, percent by mass} = \frac{100(M_1 - M_2)}{M_1 - M}$$

where

M_1 = mass of the weighing bottle with the material before heating, in g;

M_2 = mass of the weighing bottle after heating, in g; and

M = mass of the weighing bottle, in g.

ANNEX C

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF SULPHATED ASH

C-1 REAGENT

C-1.1 Concentrated Sulphuric Acid, *See* IS 266.

C-2 PROCEDURE

Weigh accurately about 2 g of the material in a tared crucible. Ignite, gently at first, until the material is thoroughly charred, cool, moisten the residue with 1 ml of sulphuric acid and ignite gently till the carbon is completely consumed. Cool the crucible in a desiccator and weigh.

NOTE — Carry out the ignition in a place protected from air currents and use as low a temperature as possible to effect the combustion of carbon.

C-3 CALCULATION

$$\text{Sulphated ash, percent by mass} = \frac{W_1}{W_2} \times 100$$

where

W_1 = mass of the residue, in g; and

W_2 = mass of the material taken for the test, in g.

ANNEX D

[*Table* 1, *Sl No.* (iv)]

DETERMINATION OF LUMIFLAVIN

D-1 REAGENTS

D-1.1 Chloroform

D-1.2 Lumiflavin

D-1.3 Reference Solution — Dissolve 25 mg of lumiflavin in 50 ml of chloroform. Dilute 1 ml of this solution with chloroform to 20 ml, and dilute 2.5 ml of the resultant solution to 100 ml. This solution contains 0.625 µg of lumiflavin/ml.

D-1.4 Test Solution

Shake 25 mg of the sample with 10.0 ml chloroform for 5 min and filter.

D-2 APPARATUS

D-2.1 Thin-Layer Chromatography

D-2.1.1 *Stationary Phase*, precoated HPTLC plates of silica gel WRF_{254}, 10 cm × 20 cm, layer thickness 0.1 mm (Merck Cat No. 1.12363).

D-2.1.2 *Mobile Phase,* water.

D-2.1.3 *Run Length*, approximately 6 cm.

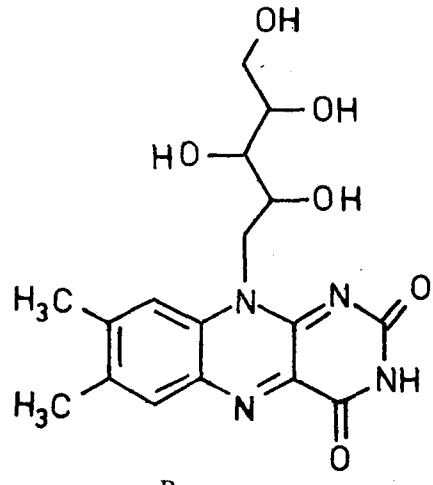
D-2.1.4 *Elution Time*, approximately 20 min.

D-3 PROCEDURE

Apply 10 ml of reference solution and 10 ml of test solution on the chromatogram and run for approximately 20 min. Dry the plate in a current of cold air and indicate the fluorescence at 366 nm.

Any spot in the chromatogram of the test solution, which corresponds to the main spot of the reference solution, shall not be larger or more intensely coloured than the reference solution spot.

(*Continued from second cover*)



RIBOFLAVIN

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act,* 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

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Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc : No. FAD 8 (1469).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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